IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | |
|---|---|
| In re application of: Beug *et al.* | Confirmation No.: |
| Appl. No. *To be assigned* | Art Unit: *To be assigned* |
| Filed: *Herewith* | Examiner: *To be assigned* |
| For: **Pharmaceutical compositions for treating tumour diseases** | Atty. Docket: 0652.1790001/EKS/BEC |

## Preliminary Amendment

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the merits of the above-identified patent application, please amend the application as follows. This Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks and arguments. 37 C.F.R. § 1.111 and MPEP 714; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent

abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

## *Amendments*

***In the Specification:***

At page 1, before the title, "Pharmaceutical compositions for treating tumour diseases," please insert the heading -- Title of the Invention--;

line 2, after the title, please insert the following section:

-- Cross Reference to Related Applications

This application is a divisional of U.S. Application No. 09/155,716, filed February 16, 1999, allowed, which is a 371 of PCT/EP97/01699, filed internationally on April 4, 1997, which was published under PCT Article 21(2) in German.--;

line 3, before "The invention relates to the field of tumour therapy," please insert the heading -- Field of the Invention--; and

line 5, please insert the heading -- Background of the Invention --.

At page 3, line 25, please insert the heading -- Summary of the Invention --.

At page 6, line 6, please insert the heading -- Detailed Description of the Invention --.

At page 31, line 24, please insert the heading -- Examples --.

At pages 30-31, please delete the Figure Summary section and replace it with the following Figure Summary section:

Figure Summary

Figs. 1A-1D: Conversion of EpRas cells into fibroblastoid cells during tumour formation in mice. Fig.1A is a schematic diagram illustrating the strategy which was used to study the epithelial-fibroblast conversion (EFC) of Ras cells *in vivo*. Fig. 1B is a photomicrograph showing cells of the clone Ep5 before subcutaneous injection. Fig. 1C is a photomicrograph showing that Ep5 cells isolated from a tumour 28 days after injection. Fig. 1D is a Southern Blot analysis of the EpRas-clone (Ep5): (i) before injection (Ep5 plastic), (ii) removed from the tumour (Ep5, tumour), and (iii) removed from the tumour and recultivated for 5 days in G418 (Ep5, ex tumour).

Figs. 2A-2H: Epithelial/mesenchymal conversion (EFC) during tumour development: time scale and behaviour of donor and receiver cells: after 3 days (Figs. 2A and 2E), 7 days (Figs. 2B and 2F), 15 days (Figs. 2C and 2G)

and 28 days (Fig. 2H). Fig. 2D is a photomicrograph showing parental EpH4 cells 15 days after subcutaneous injection.

Figs. 3A-3D: Organogenesis and epithelial polarity are destroyed by serum or TGFβ1. Figs. 3A is a photomicrograph showing Ep4H cells in a serum-free collagen gel. Fig. 3B is a photomicrograph showing EpRas cells in a serum-free collagen gel. Fig. 3C is a photomicrograph showing cells after the addition of 10% FCS. Fig. 3D is a photomicrograph showing EpRas cells grown with TGFβ1 (5 ng/ml).

Figs. 4A-4F: TGFβ1 destroys the cell polarity in Ras-transformed breast epithelial cells. Fig. 4A is a transmission electron micrograph of EpRas cells in serum-free collagen gel. Fig. 4B is a photomicrograph of a frozen section through an alveolar cyst formed by EpRas cells in serum-free collagen gel. Fig. 4C is a photomicrograph of a Lowicryl section through an alveolar cyst formed by EpRas cells in serum-free collagen gel. Fig. 4D is a transmission electron micrograph of disordered strings of EpRas cells after treatment with TGFβ1. Fig. 4E is a photomicrograph of a frozen section through disordered strings of EpRas cells after treatment with TGFβ1. Fig. 4F is a photomicrograph of a Lowicryl section through disordered strings of EpRas cells after treatment with TGFβ1.

Figs 5A-5D: Fibroblastoid EpRas cells are highly invasive in the chicken embryo heart invasion assay. Figs. 5A-5D are photomicrographs of *in vivo* fluorescence-labeled cells co-cultured for 7 days with chicken embryo heart fragments: non-tumorogenic epithelial starting cells (EpH4 cells)

(Figs. 5A-5B), non-converted epithelial EpRas cells (Fig. 5C), and converted fibroblastoid cells after TGFβ1 treatment (Fig. 5D).

Figs. 6A-6F: TGFβ1 maintains the fibroblastoid phenotype of converted EpRas cells via an autocrine loop. Figs. 6A-6D are photomicrographs of a cell clone, from fibroblastoid cells isolated from a tumour and grown in medium containing 1% FCS, on day 1 (Fig. 6A), day 3 (Fig. 6B), day 5 (Fig. 6C) and day 10 (Fig. 6D). Figs. 6E-6F are photomicrographs of the same cells after a further 8 days in collegian gels, in the absence (Fig. 6E) and in the presence (Fig.6F) of TGFβ1 neutralising antibodies.

Figs. 7A-7B: Converted EpRas cells produce high concentrations of TGFβ1. Fig. 7A shows a semi-quantitative PCR analysis for TGFβ1-mRNA. Fig. 7B show TGFβ1 concentrations in cell culture supernatants as measured by Western Blot and ELISA.

Figs. 8A-8F: TGFβ1 triggers the transition from the epithelial to the fibroblastoid state as well as the invasiveness of the cells in experimentally induced tumours. Figs. 8A-8B are photomicrographs of frozen sections of a tumour on day 4. Figs. 8C-8D are photomicrographs of frozen sections of a tumor on day 15. Fig. 8E is a photomicrograph of EpRas cells injected subcutaneously into nude mice without 3-Elvax Slow Release Pellets charged with recombinant (active) TGFβ1. Fig. 8F is a photomicrograph of EpRas cells injected subcutaneously into nude mice with 3-Elvax Slow Release Pellets charged with recombinant (active) TGFβ1.

Fig. 9: Model for the activity of TGFβ1 in tumour development.

Figs. 10A-10D: TGFβ1 induced *in vitro* morphogenesis and apoptosis in normal mammary gland epithelial cells. Fig. 10A is a photomicrograph showing that, in the absence of additional TGFβ1, the cells do not form any tubular structures. Fig. 10B is a photomicrograph showing that, in the presence of 0.1 ng/ml of TGFβ1 the cells form branched structures, but these branched structures lack lumina. Fig. 10C (lower magnification on the left, higher magnification on the right) is a photomicrograph showing that higher concentrations of TGFβ1 cause cell death (apoptosis). Fig. 10D is a photomicrograph showing that, if the TGFβ1 is removed, on day 7 by washing, from cultures having the structures shown in Fig. 10C, the cells form distinct hollow structures.

Fig. 11: *In vivo* expression of TGFβ1 during the formation of the normal breast.

Fig. 12: *In vivo* expression of TGFβ1 during the breakdown of the fully developed mammary gland after ablactation.

Fig. 13: Inhibition of the invasivity of human tumour cells by TGFβ-neutralising antibodies.

Fig. 14: Expression of a dominant-negative TGFβ-receptor (TβRII-dn) in Ras-transformed breast epithelial cells inhibits EFC and tumour growth.

Fig. 15: Expression of TβRII-dn in mouse-colon carcinoma calls (CT26) prevents growth in collagen gels and invasivity *in vitro*.

Fig. 16: Expression of TβRII-dn in CT26 cells inhibits metastasisation *in vivo*.

Fig. 17: TβRII-dn expressing CT26 cells are incapable of forming metastases in the lung even after intravenous injection.

Fig. 18: Expression of a PAI-1-promoter-reporter construct in CT26- and CT26-TβRII-dn cells.

***In the Claims:***

Please cancel claims 1-15 without prejudice to or disclaimer of the subject matter therein.

Please add the following new claims 16-24:

16. (new) A pharmaceutical composition comprising as an active compound a substance which inhibits the activity of TGFβ on tumour cells of epithelial origin, wherein said agent is present in an amount effective for the treatment of epithelial, invasive tumour diseases, wherein said diseases are characterised by tumour cells having a reversible transition from an epithelial, non-invasive state into an invasive state, and said agent is not a TGFβ antisense oligonucleotide.

17. (new) The pharmaceutical composition according to claim 16, comprising as an additional active compound a second substance which inhibits, in said tumour cells, at least one of (a) the expression or function of oncogenic Ras, (b) the overexpression of normal Ras, or (c) the activation of normal Ras by receptor-tyrosinekinase.

18. (new) The pharmaceutical composition according to claim 17, wherein said second active compound directly inhibits the activation of Ras.

19. (new) The pharmaceutical composition according to claim 17, wherein said second active compound indirectly inhibits the activation of Ras.

20. (new) The pharmaceutical composition according to claim 19, wherein said second active compound is an inhibitor of a receptor-tyrosinekinase.

21. (new) The pharmaceutical composition according to claim 20, wherein said second active compound is an inhibitor of the EGF receptor.

22. (new) A method for treating tumour diseases comprising administering to a human the pharmaceutical composition of claim 16, thereby changing established, invasive tumour cells back into a non-invasive, epitheloid state.

23. (new) The method according to claim 22, wherein said method is for treating breast tumours.

24. (new) The method according to claim 22, wherein said method is for treating kidney cell carcinomas.

## *Remarks*

The specification has been amended to include the proper section headings according to MPEP § 608.01(a). The specification has also been amended to reflect the present status of applications pertinent to the Cross Reference to Related Applications. The specification has been amended in the Figure Summary, at pages 30, lines 13-35, to include descriptions of subfigures 1A-1D, 2A-2H, 3A-3D, 4A-4F, 5A-5D, 6A-6F, 7A-7B, 8A-8F and 10A-10D. Support for this amendment can be found at page 42, line 15, through page 60, line 18, of the application as filed. The Figure Summary has also been amended at pages 30-31 to correct certain typographical errors.

Upon entry of the foregoing amendment, claims 16-24 are pending in the application, with claim 16 being the independent claim. Claims 1-15 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 16-24 are sought to be added. Support for new claims 16-24 can be found throughout the specification and in the original claims.

### *Election/Restriction Requirement*

In the parent (application no. 09/155,716, § 371 date February 16, 1999) the Examiner made an election/restriction requirement, perceiving two inventions (*see* File Wrapper Paper No. 7, mailed November 8, 1999, paragraph numbers 2 and 3). New claims 16-24 are directed to the subject matter of the previously non-elected claims.

***Conclusion***

Applicants respectfully submit that no new matter has been added by way of the above amendments. Applicants request that these amendments be entered into the application prior to examination.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Bruce E. Chalker

Bruce E. Chalker
Attorney for Applicants
Registration No. 47,480

Date: February 26, 2002

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

## Version with markings to show changes made

***In the Specification:***

Various section headings have been added at pages 1, 3, 6 and 31.

A "Cross Reference to Related Applications" sections has been added at page 1.

At pages 30-31, the Figure Summary section has been amended as follows:

Figure Summary

[Fig. 1]

Figs. 1A-1D: [conversion] Conversion of EpRas cells into fibroblastoid cells during tumour formation in mice. Fig.1A is a schematic diagram illustrating the strategy which was used to study the epithelial-fibroblast conversion (EFC) of Ras cells *in vivo*. Fig. 1B is a photomicrograph showing cells of the clone Ep5 before subcutaneous injection. Fig. 1C is a photomicrograph showing that Ep5 cells isolated from a tumour 28 days after injection. Fig. 1D is a Southern Blot analysis of the EpRas-clone (Ep5): (i) before injection (Ep5 plastic), (ii) removed from the tumour (Ep5, tumour), and (iii) removed from the tumour and recultivated for 5 days in G418 (Ep5, ex tumour).

[Fig. 2]

Figs. 2A-2H: [epithelial/mesenchymal] Epithelial/mesenchymal conversion (EFC) during tumour development: time scale and behaviour of donor and receiver cells; after 3 days (Figs. 2A and 2E), 7 days (Figs. 2B and 2F), 15 days (Figs. 2C and 2G) and 28 days (Fig. 2H). Fig. 2D is a photomicrograph showing parental EpH4 cells 15 days after subcutaneous injection.

[Fig. 3]

Figs. 3A-3D: [organogenesis] Organogenesis and epithelial polarity are destroyed by serum or TGFβ1. Figs. 3A is a photomicrograph showing Ep4H cells in a serum-free collagen gel. Fig. 3B is a photomicrograph showing EpRas cells in a serum-free collagen gel. Fig. 3C is a photomicrograph showing cells after the addition of 10% FCS. Fig. 3D is a photomicrograph showing EpRas cells grown with TGFβ1 (5 ng/ml).

[Fig. 4]

Figs. 4A-4F: TGFβ1 destroys the cell polarity in Ras-transformed breast epithelial cells. Fig. 4A is a transmission electron micrograph of EpRas cells in serum-free collagen gel. Fig. 4B is a photomicrograph of a frozen section through an alveolar cyst formed by EpRas cells in serum-free collagen gel. Fig. 4C is a photomicrograph of a Lowicryl section through an alveolar cyst formed by EpRas cells in serum-free collagen gel. Fig. 4D is a transmission electron micrograph of disordered strings of EpRas cells after treatment with TGFβ1. Fig. 4E is a photomicrograph of a frozen

section through disordered strings of EpRas cells after treatment with TGFβ1. Fig. 4F is a photomicrograph of a Lowicryl section through disordered strings of EpRas cells after treatment with TGFβ1.

[Fig. 5]

Figs 5A-5D: [fibroblastoid] Fibroblastoid EpRas cells are highly invasive in the chicken embryo heart invasion assay. Figs. 5A-5D are photomicrographs of *in vivo* fluorescence-labeled cells co-cultured for 7 days with chicken embryo heart fragments: non-tumorogenic epithelial starting cells (EpH4 cells) (Figs. 5A-5B), non-converted epithelial EpRas cells (Fig. 5C), and converted fibroblastoid cells after TGFβ1 treatment (Fig. 5D).

[Fig. 6]

Figs. 6A-6F: TGFβ1 maintains the fibroblastoid phenotype of converted EpRas cells via an autocrine loop. Figs. 6A-6D are photomicrographs of a cell clone, from fibroblastoid cells isolated from a tumour and grown in medium containing 1% FCS, on day 1 (Fig. 6A), day 3 (Fig. 6B), day 5 (Fig. 6C) and day 10 (Fig. 6D). Figs. 6E-6F are photomicrographs of the same cells after a further 8 days in collegian gels, in the absence (Fig. 6E) and in the presence (Fig.6F) of TGFβ1 neutralising antibodies.

[Fig. 7]

Figs. 7A-7B: [converted] Converted EpRas cells produce high concentrations of TGFβ1. Fig. 7A shows a semi-quantitative PCR analysis for TGFβ1-mRNA. Fig. 7B show TGFβ1 concentrations in cell culture supernatants as measured by Western Blot and ELISA.

[Fig. 8]

Figs. 8A-8F: TGFβ1 triggers the transition from the epithelial to the fibroblastoid state as well as the invasiveness of the cells in experimentally induced tumours. Figs. 8A-8B are photomicrographs of frozen sections of a tumour on day 4. Figs. 8C-8D are photomicrographs of frozen sections of a tumor on day 15. Fig. 8E is a photomicrograph of EpRas cells injected subcutaneously into nude mice without 3-Elvax Slow Release Pellets charged with recombinant (active) TGFβ1. Fig. 8F is a photomicrograph of EpRas cells injected subcutaneously into nude mice with 3-Elvax Slow Release Pellets charged with recombinant (active) TGFβ1.

Fig. 9: [model] Model for the activity of TGFβ1 in tumour development.

[Fig. 10]

Figs. 10A-10D: TGFβ1 induced *in vitro* morphogenesis and apoptosis in normal mammary gland epithelial cells. Fig. 10A is a photomicrograph showing that, in the absence of additional TGFβ1, the cells do not form any tubular structures. Fig. 10B is a photomicrograph showing that, in the presence of 0.1 ng/ml of TGFβ1 the cells form branched structures, but these branched structures lack lumina. Fig. 10C (lower magnification on the left, higher magnification on the right) is a photomicrograph showing that higher concentrations of TGFβ1 cause cell death (apoptosis). Fig. 10D is a photomicrograph showing that, if the TGFβ1 is removed, on day 7 by washing, from cultures having the structures shown in Fig. 10C, the cells form distinct hollow structures.

Fig. 11: *In vivo* expression of TGFβ1 during the formation of the normal breast.

Fig. 12: *In vivo* expression of TGFβ1 during the breakdown of the fully developed mammary gland after ablactation.

[Fig. 13.]

Fig. 13: [inhibition] Inhibition of the invasivity of human tumour cells by TGFβ-neutralising antibodies.

Fig. 14: [expression] Expression of a dominant-negative TGFβ-receptor (TβRII-dn) in Ras-transformed breast epithelial cells inhibits EFC and tumour growth.

Fig. 15: [expression] Expression of TβRII-dn in mouse-colon carcinoma calls (CT26) prevents growth in collagen gels and invasivity *in vitro*.

Fig. 16: [expression] Expression of TβRII-dn in CT26 cells inhibits metastasisation *in vivo*.

Fig. 17: TβRII-dn expressing CT26 cells are incapable of forming metastases in the lung even after intravenous injection.

Fig. 18: [expression] Expression of a PAI-1-promoter-reporter construct in CT26- and CT26-TβRII-dn cells. [ ]

***In the Claims:***

Claims 1-15 have been canceled.

Claims 16-24 have been added.